



Isolation, Identification, and Prevalence of Bacterial Pathogens in Patients with Second and Third Degree Burns

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ABSTRACT

Background: Second and third-degree burn wounds are an ideal environment for colonization by bacterial pathogens due to damage to the skin barrier, where *S. aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* predominate. They are characterized by high resistance to antibiotics and the ability to form biofilms, which increases infection and sepsis rates. Therefore, early diagnosis of pathogens can facilitate rapid recovery and prevent them from penetrating the body.

Objectives: This study aimed to determine the prevalence and distribution of bacterial pathogens in burn wounds at our center to inform local infection control and empiric treatment protocols.

Methodology: Cross-sectional study of 60 patients, ages ranging from 1 to 60 years, for both sexes. Swabs were processed via culture on standard and selective media, with identification via biochemical tests and VITEK-2.

Results: All patients suffered from second and third degrees of burns, and most of them were women at a rate higher than that of men. As for the age group, 21–30 had the highest percentage of burned people under study compared to the rest of the age groups. 44 bacterial isolates were identified, 29 isolates from patients with second-degree burns and 15 isolates from patients with third-degree burns, including 19 isolates of *Pseudomonas aeruginosa*, 13 isolates of *Acinetobacter baumannii*, and 9 isolates of *Staphylococcus aureus*. At the same time, there were 3 isolates of *Klebsiella pneumoniae* bacteria.

Conclusion: Early detection prevents the progression of local wound infections to septic shock, the leading cause of death in burn units. These types of bacteria, known as hospital-acquired bacteria, possess complex defense mechanisms and are classified as multidrug-resistant. Their presence in the burn area often leads to skin graft failure, as they inhibit the growth of new cells responsible for adhesion. Furthermore, knowing this allows doctors to choose the appropriate antibiotic instead of relying on guesswork, thus reducing the exacerbation of resistance.

Keywords: burns, nosocomial infection, opportunistic bacteria.

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INTRODUCTION

In patients with severe burns, one of the major cause of morbidity and mortality is bacterial burn wound infections. The primary cause of increased morbidity and death in burn patients is microbiological infection, particularly infections brought on by multiple drug-resistant organisms (MDRO) (Chen et al., 2020). Globally, burn injuries account for approximately 26,000 deaths annually, making them one of the most common public health concerns. Burn injuries are injury to the skin or tissue brought on by exposure to chemicals, heat, or electricity, among other factors (Tanzin et al., 2022).

Depending on the location and amount of time since the injuries, different organisms can cause burn wound infections, and the diagnosis is complex. Pathogens that are still most prevalent are *Staphylococcus* and *Pseudomonas* species (D'Abbondanza and Shahrokhi, 2021). Burn patients who sustain second- or third-degree burns over a period of time and who develop infections tend, on average, to have a higher total body surface area (TBSA), higher Burn Severity Index (ABSI) scores, and longer hospital stays (Tsolakidis et al., 2022).

Burn patients are at high risk for *A. baumannii* infection from internal sources. It has been isolated from burn patients and healthcare workers (HCWs) and poses a serious global health threat (Firoozeh et al., 2023). Burn patients who sustain second- or third-degree burns over a period of time and who develop infections tend, on average, to have a higher total body surface area (TBSA), higher Burn Severity Index (ABSI) scores, and longer hospital stays (Tsolakidis et al., 2022). Burn patients are at high risk for *A. baumannii* infection from internal sources. It has been isolated from burn patients and healthcare workers (HCWs) and poses a serious global health threat (Firoozeh et al., 2023).

Clearly state that while global data exists, local epidemiological patterns vary significantly due to hospital flora and practices. The opportunistic

pathogen *Klebsiella pneumoniae* is responsible for a high death rate in burn victims and a wide range of illnesses in both humans and animals (Hateet, 2021).

AIMS OF THE STUDY

This study aimed to determine the prevalence and distribution of bacterial pathogens in burn wounds at our center to inform local infection control and empiric treatment protocols.

METHODOLOGY

In a cross-sectional study conducted at the laboratory Public Health Central in al-Najaf al-Ashraf, 60 skin swabs were obtained from inpatients with 2nd/3rd degree burns and were treated at the burn center for the period from October 2024 to January 2025. There were no exclusion criteria during sampling based on burn age or whether patients were already taking medications for systemic diseases. The samples were gathered from both genders and all age groups.

Identification and Isolation of Bacteria

According to Montazeri et al. (2020), the isolation, diagnosis, and characterization of bacteria from burn injuries included morphological traits of culture media, microscopy, biochemical identification, and the final confirmatory examination is by VITEK 2 compact. Following their immediate inoculation in Cary Blair (IVD products, China) and transportation to the laboratory, the burn swabs were first isolated by striking them with nutrient broth, MacConkey's agar, and blood agar (Himedia, India) base containing human blood. They were then subcultured on selective media, such as mannitol salt agar (Oxoid, England) for *S. aureus*. Pure colonies after subculture was followed by microscopic examination after staining. with Gram stain for bacteria to determine the shape, size, and arrangement of the microscopic cells under the microscope. Further biochemical examinations were conducted for the microscopically

diagnosed bacteria which includes testing for oxidase, catalase, slide coagulation, and urease and then confirmed by VITEK examination.

RESULTS

Patient Profile for Those Suffering from Burn Injuries

Burn skin swabs from 60 patients served as the samples. People between the ages of one and sixty were the subjects of the samples. There were 37 female patients and 23 male patients, representing both genders. Along with bacterial infections, the majority of the patients had second- and third-degree burns.

Age-Group-Based Distribution of Burn Infections

It is worth noting, according to the included age range from 1 year to 60 years with both male and females Figure (1) indicated the highest rate of burns by age was between 21-30 years (33.3%), followed by age group 11-20 (18.3%), after that the age group 1-10 (16.6%), then the age group 31-40 (15%), while the least frequented groups were in the age group 41-50 (11.6%) and age group 51-60 (5%). These results indicated a significant difference among age groups. Figure (1).

Disruption of Gender in Burn Patients

In the current study, the burns spread more in females at higher rates (37/60, 61.6%) compared to males (23/60, 38.3%). Figure (2).

Based on percentage, the ages of the burn patients ranged in both sexes from 1 to 60, as shown in Table (1).

Degree of Burns Among Patient States

All cases in the current research were with the second and third degrees of burns. The second degree was (65%) and the third degree was (25%), While there were patients whose burns were mixed between second and third degree. Table (2).

Isolation and Identification of Bacteria"

Isolation and Diagnosis of *Pseudomonas Aeruginosa*

As a facultative aerobic rod that is gram-negative, non-fermentative, non-sporulating, and motile via polar flagellum, *Pseudomonas aeruginosa* is one of the most significant and opportunistic infections that cause a high risk of death and morbidity in hospitalized patients with weakened immune systems. In the current study, *Pseudomonas aeruginosa* was isolated after 24 hours at 37°C, and small colonies were observed; on nutrient agar, it produced a grape-like odor with yellow-green pigment. The agar, combined with pyocyanin, was also cultured on MacConkey and produced non-lactose fermenting colonies. However, on blood agar, it produced hemolysis. *Pseudomonas aeruginosa* appeared in gram-negative rods under the microscope and also showed an oxidase and catalase positive result, after which it was confirmed by VITEK 2 compact as *P. aeruginosa* with a probability of 97%.

Isolation and Diagnosis of *Acinetobacter Bumanni*

The investigation demonstrated that after overnight growth, *Acinetobacter baumannii* formed a complex streak on MacConkey agar at 44° C and appeared small, pale, colorless, round, and regular, not fermented to lactose. On blood agar, *Acinetobacter baumannii* appeared in white or light gray and was convex in shape if it did not seem to have decomposition areas around the developing colonies due to its inability to produce hemolysis. *Acinetobacter baumannii* appeared as gram-negative coccobacilli under the microscope but showed oxidase-negative and catalase-positive results after that confirmed by VITEK 2 compact as an *A. baumannii* with a probability of 99%.

Isolation and Diagnosis of *Staphylococcus Aureus*

Using conventional standard bacteriological and biochemical tests, *S. aureus* isolates from patients with burns were determined. All the specimens cultured on blood agar medium supplemented with 5% human blood at a temperature of 37°C under aerobic conditions for primary isolation were also selected by using selective media mannitol salt agar (MSA) for confirmation of the mannitol fermentation. *S. aureus* colonies that were shown were large, round, opaque, and golden yellow in color. Furthermore, it showed a β -hemolysis zone on blood agar and the ability to ferment mannitol on MSA. After culturing *S. aureus* isolates, they were subjected to Gram staining, catalase, oxidase, and further slide coagulase tests. Isolated isolates were classified as gram-positive, small, spherical, cluster-like grapes, oxidase-negative, and catalase-positive. Also, isolates showed positive results with a slide coagulase test as an identification diagnosis and confirmed with VITEK 2 compact with a probability of 93%.

Isolation and Diagnosis of *Klebsiella pneumonia*

Klebsiella pneumonia isolated in the current study on MacConkey agar was rose pink colonies due to lactose fermentation, huge dome-shaped structure, and mucoid, caused by an extracellular slime layer. Under the microscope, *Klebsiella pneumonia* was gram-negative, rod-shaped, short, fat, and straight. The biochemical test characteristics that were discovered were as follows: catalase positive, oxidase negative, and urease positive. After that, confirmed by VITEK 2 Compact with a probability 99%.

Prevalence of bacterial infection in burns patients

An interesting result in this data is that out of 60 burn swab samples, 44/60 (73.3%) were bacterial isolates; among these, *Pseudomonas aeruginosa* was highly prevalent (19/44, 43.1%), *Acinetobacter baumannii* was 13/44 (29.5%), *Staphylococcus*

aureus was 9/44 (20.4%), and *Klebsiella pneumonia* was 3/44 (6.8%). A is shown in Table (3) and Figure (3).

Types of Bacteria Isolated According to The Ages of Patients

According to what was mentioned in our findings, burn injuries were the most common in the ages between 21 and 30. In addition to that, Table (4) highlights the bacterial types isolated from each age group. Four bacterial types recorded high rates: (90.9%), (88.8%), (80%), and (60%) in age groups 11–20, 31–40, 21–30, and 1–10, respectively. While recorded low rates in age groups 41–50 and 51 – 60.

Degree of burns, bacteria types among patient states

Also, patient cases in our research were divided according to the types of bacteria associated with burn degrees, as in Table (5).

Generally, in the current study, there has been growth and high levels of bacteria: *Pseudomonas aeruginosa* (68.4%), *Acinetobacter baumannii* (69.2%), and *Staphylococcus aureus* (66.6%) in second-degree burns. These bacteria are considered opportunistic bacterial pathogens primarily associated with hospital-acquired infections, while *Klebsiella pneumonia* recorded higher growth rates (66.6%) in third-degree burns than in second-degree burns (33.3%) because it can display high degrees of virulence and antibiotic resistance. Figure (4) showed that.

DISCUSSION:

Patient Profile for Those Suffering from Burn Injuries

In our current research, when asking questions to collect information, the answers were mostly conservative for both genders. At the same time, the reasons were clear due to the nature of the majority's work, which ages between 21 and 50 years old for mothers in the kitchen, baking pastries and bread,

and the harsh nature of the work for men. Curiosity in children under 5 years old can lead to accidents such as touching the hot surface of the oven or heater or spilling hot liquids like boiling water or tea. As for the elderly, aged 55 and over, their injuries included burns resulting from smoking and related fires, such as burning mattresses and blankets while smoking in bed, accompanied by skin thinning, weakened senses, and limited mobility, or burns from hot water taps. A previous study reported in their report on suicide that young women are more represented in suicide attempts and thinking about using potentially fatal methods such as self-immolation as a result of high rates of depression and societal and domestic violence (Younis and Lafta, 2023).

Age-Group-Based Distribution of Burn Infections

This is in line with this study, which shows that older individuals aged 18 to 59 years are more affected by burns than others (Cunha et al., 2023). In addition, research has shown that the largest percentage of burn patients were in the age group > 18 years (Setiawan and Haryono, 2021).. However, other studies indicate that children are most affected. This was found in the research conducted by (Chen et al., 2021). Another study recorded that the majority of patients were in the age group from 0 to 10 years (Trakroo et al., 2023).

Disruption of Gender in Burn Patients

Our current research findings were consistent with other research where females had significantly more frequent burns (Sadaka and Abdeldaim, 2024). Worldwide, women may suffer more serious burn injuries than men. In low- and middle-income countries, gender disparities are particularly noticeable (Mehta et al., 2022).

Degree of Burns Among Patient States

Burn severity can be classified into burn degrees according to the layers of skin damage (Suha and Sanam, 2022). Another research study

recorded that 73.68% of people suffered from second-degree burns (Putri and Yanuardani, 2023). A third-degree burn, also known as a complete burn, is the most serious and affects the layers of skin and dermis. It also extends to the subcutaneous tissue. The nerves located at this depth are also damaged, which leads to the patient not feeling any sensation or pain. Which makes it more susceptible to infections due to bacteria (Warby and Maani, 2023). Longer hospital stays raise the likelihood of contracting such drug-resistant illnesses, and hospital-acquired infections are a major concern for patients who are admitted. The three most prevalent multidrug-resistant (MDR) Gram-negative bacteria found in burn wound infections (BWIs) are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Roy et al., 2024). Their high level of resistance is mostly due to their capacity to build biofilm (Scoffone et al., 2025). LPS strongly stimulates the immune system response. Dysregulated inflammatory responses result from this, ultimately raising morbidity and death (Jaafar et al., 2023).

Isolation and Diagnosis of *Pseudomonas Aeruginosa*

The pathogen *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a variety of infections. Particularly in burn patients (Jaafar et al., 2023). *P. aeruginosa* produces at least 4 distinct pigments; pyocyanin- bluish-green, pyoverdine- yellow-green, gives color to pus and gives a greenish color to the agar when combined with pyocyanin, pyorubine-red, and pyomelanin- black (Daher., 2024). A previous study reported the gold standard is VITEK VR –MS for the quick and reliable identification of *Pseudomonas*-associated nosocomial infections in critically sick patients admitted to the intensive care unit (Moehario et al., 2021). Moreover, microscopic inspection, biochemical testing, and the VITEK-2 compact system were used to identify *P. aeruginosa* bacteria from a variety of sources in Baghdad

hospitals (Al-fridawy et al., 2020). Both conventional techniques and the VITEK2 system were used to identify and diagnose *P. aeruginosa* isolates from patients suffering from burn wounds and multiple hospitals in Baghdad (Daher., 2024).

Isolation and Diagnosis of Acinetobacter Baumanni

Acinetobacter baumannii is a bacterial pathogen that is commonly linked with hospital-acquired illnesses (Centers for Disease Control and Prevention, 2019). Septicemia, wound infections, pneumonia, and urinary tract infections are all caused by *Acinetobacter baumannii*, which is a major hazard to hospitalized patients (Nguyen and Joshi, 2021). However, MacConkey and blood agar plates were used to isolate *Acinetobacter* (Kumar et al., 2020). Another research study identified bacteria *Acinetobacter baumannii* from people in serious condition in the hospital by using several culture media in order to compared between them. Identification of *A. baumannii* isolates by implementing special selective media such as blood agar and MacConkey agar (Ahmad et al., 2020). Among several studies, *Acinetobacter*, as it is now defined, are gram-negative bacteria that are strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, and oxidase-negative (Stanley et al., 2023). A Previous study also performed VITEK 2 compact for identification of *Acinetobacter baumannii* (Michael et al., 2023).

Isolation and Diagnosis of Staphylococcus Aureus

The current isolation method looks similar; implicated swabs from patients and healthcare workers in a tertiary medical center's burn unit in Ghana were streaked on 5% sheep blood agar (BA) and incubated overnight at 37°C (Amissah et al., 2017). Deep partial (DPT) and full-thickness (FT) burn wounds colonized with *Staphylococcus aureus*. Damage-associated molecular patterns (DAMPs)

changed dynamically as a result of *S. aureus* infection. These variations in DAMPs are thought to be linked to the severity of the burn and the bioburden of *S. aureus*. Overall, this model demonstrates *S. aureus* evasiveness by suppressing the immune response, allowing it to thrive in the burn site (Weaver et al., 2021). *S. aureus* was also cultured on Mannitol Salt Agar (MSA) and isolated to ferment mannitol and gram staining (Budiarto et al., 2019). A coagulase test was used to confirm the diagnosis of *S. aureus* (MacFaddin, 2000; Mayar Hezam, 2019). Prior research used the VITEK-2 small GP colorimetric identification card (BioMérieux, France) to identify *S. aureus* isolates from burn victims in a regional burn hospital in Southeastern China (Chen et al., 2018).

Isolation and Diagnosis of Klebsiella pneumonia

The most prevalent cause of hospital-acquired pneumonia nowadays is *K. pneumoniae* pneumonia which can enter the body through burn wounds during hospital treatment. Human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract are commonly colonized by *Klebsiella pneumonia*. Once within the body, the bacteria can exhibit significant levels of virulence and drug resistance (Kot et al., 2023). Hassan (2021) recorded in his investigation that *Klebsiella pneumoniae* colonies are pink due to lactose fermentation, which resembles our findings. In a registered study in India, it was reported that the VITEK-2 Compact (Biomérieux) was used in the research of isolates obtained from various clinical samples in a tertiary care hospital (Kaur et al., 2019).

Prevalence of bacterial infection in burns patients

The prevalence of microbial infections is very dynamic and varied according to geographic area, sampling and culturing methods, and other confounding factors. Another study in the burn unit at Imam al-Sadiq Hospital (peace be upon him) in Babylon concluded that the prevalence of *P. aeruginosa* bacteria was high among clinical samples

(Fakhry and Aljanabi, 2024). Our current results appear to be very close to what was obtained from Forson et al., (2017), whose results were *Pseudomonas* sp. (30.2 %) and *Acinetobacter* sp. We're the most often isolated species (20.9 %). and *Staphylococcus aureus* (2.3%) was the least commonly isolated. In another study in Iraq, in the burn unit at Al-Sadr Teaching Hospital in the city of Maysan, distinct bacterial isolates were identified, including *Pseudomonas aeruginosa*, *Staphylococcus*, and *Klebsiella*, and *Pseudomonas* was the most common pathogen (Hateet, 2021). The current findings are in agreement with D'Abbondanza and Shahrokhi (2021), who reported that the most common pathogens in burns remain *Staphylococcus* and *Pseudomonas* spp. While not in agreement with the research submitted by Montazeri et al., (2020) from the burn hospital of Yazd in Iran, where the dominant genus of G+ve bacteria was *Staphylococcus epidermidis* (62.5%), and the dominant genus of G-ve bacteria was *Citrobacter freundii* (11.5%). Another study in Iraq included detecting *Staphylococcus aureus* isolates found in (67%), collected from eight burn units in Baghdad throughout the examination (Abdulrahman et al., 2020).

Types of Bacteria Isolated According to The Ages of Patients

Another study agreed with our results from the Burn Care Unit of a tertiary care hospital in Jharkhand, India reporting that a bacterial infection was identified in 61.87% of the 16–30 and 31–45-year-old age groups. In addition to that *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were discovered to be the most common organisms in patients (Gupta et al., 2019).

Degree of burns, bacteria types among patient states

The previous study recorded that each *Pseudomonas aeruginosa*, *Acinetobacter baumannii*,

Klebsiella pneumonia, and *Staphylococcus aureus* can cause hospital-acquired infections. There is a relationship between burns and bacterial infection. Significant thermal burns cause immunosuppression, which makes burn victims more susceptible to infection (Baj et al., 2020). Also, the samples in our research were all second-and third-degree burns, and this is the cause of the bacterial infection and its ease of penetration, especially after losing the first line of defense, which is the skin. Also, pre-hospital treatment on the scene is critical for decreasing the severity of severe burn injuries and protecting against bacterial infections, particularly those of the second and third degrees (He et al., 2021).

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It is worth noting that the current study was characterized by a small sample size, a design that was limited to one center, the lack of data on antibiotic resistance, and the absence of correlation between clinical outcomes.

CONCLUSIONS:

To stop infections from spreading and make sure the right medication is chosen, it is essential to monitor bacterial colonization. This will help prevent the development of multi-resistant bacteria. It is critical for practitioners to appropriately choose, dose, and provide antibiotics to burn patients to determine the best course of care.

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TABLES & Figures:

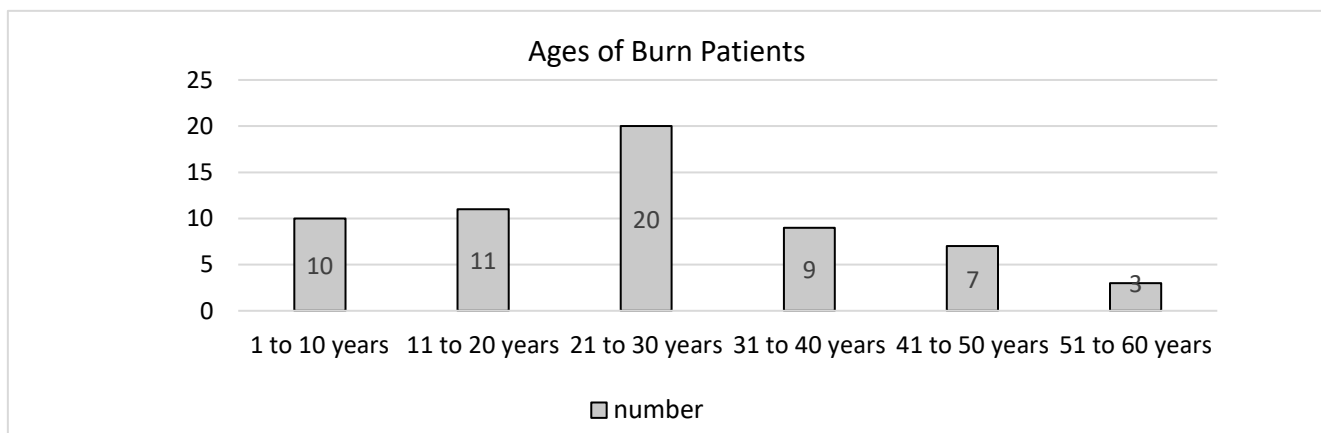


Figure (1): Range of Ages of Patients with Burns

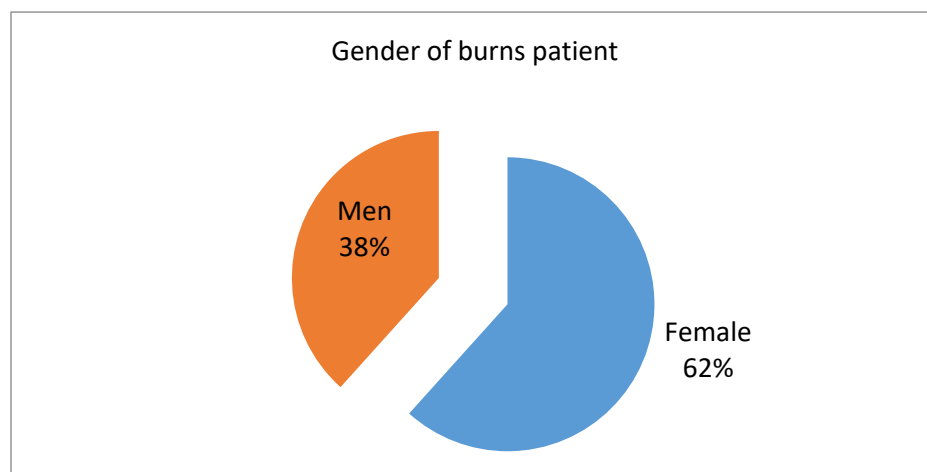


Figure (2): Percentage of The Burn Patients According Gender

Table (1): Distribution of Patients According to Age and Gender

Age	1- 10 (%)	11 - 20 (%)	21 - 30 (%)	31 – 40 (%)	41 – 50 (%)	51 – 60 (%)	Total number & Percentage
Gender							
Female	6 (10)	8 (13.3)	13 (21.6)	5 (8.3)	3 (5)	2 (3.3)	37 (62%)
Male	4 (6.6)	3 (5)	7 (11.6)	4 (6.6)	4 (6.6)	1 (1.6)	23 (38%)
Total number	10	11	20	9	7	3	60
Percentage	16.6	18.3	33.3	15	11.6	5	100%

Table (2): Distribution of The Burn Patients According to Degree of Burn

Degree	Thickness	Frequency	Percentage %	Mix of 2nd and 3rd degree Total (%)
Second	Superficial partial	39	65	6 (10)
Third	Deep partial	15	25	

Table (3): Types of Bacteria, Number of Samples, and Percentage of Bacteria Isolates

Bacterial isolates	Frequency N= 44	Percentage %
<i>Acinetobacter baumannii</i>	13	13/44 (29.5)
<i>Klebsiella pneumonia</i>	3	3/44 (6.8)
<i>Pseudomonas aeruginosa</i>	19	19/44 (43.1)
<i>Staplylococcus aureus</i>	9	9/44 (20.4)
Total isolates	44	100

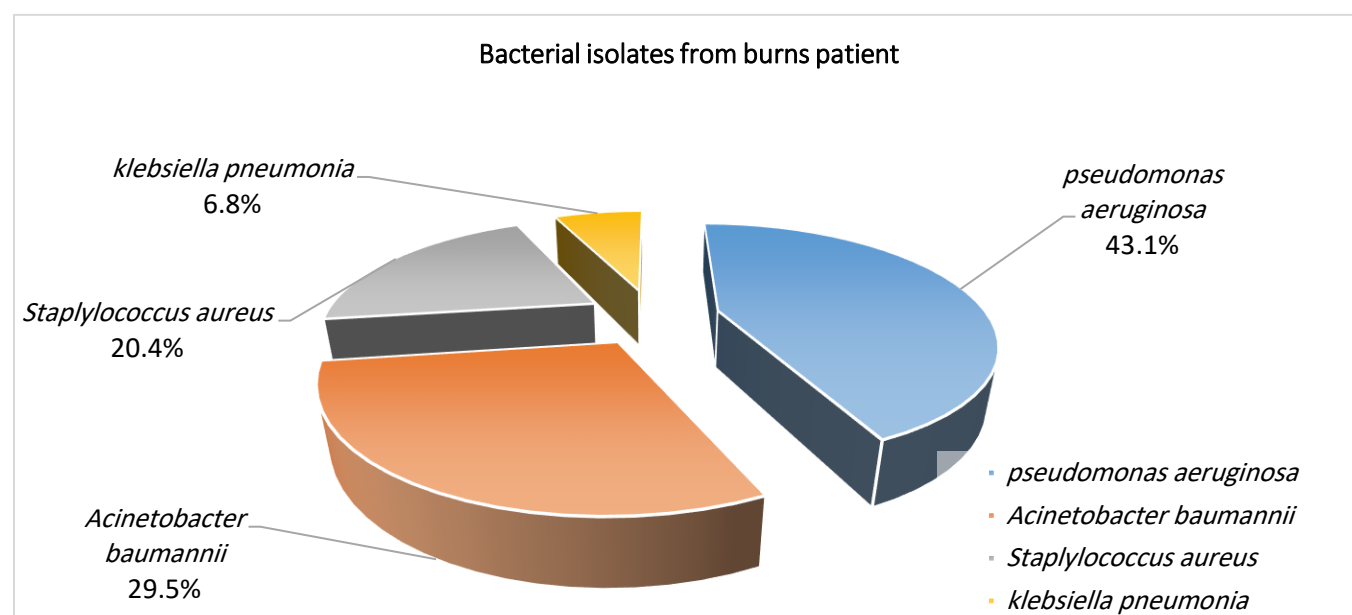


Figure (3): Bacterial Isolates from Skin Burn Patients

Table (4): Distribution of Pathogens by Age Groups

Age Bacteria	1- 10 Total=10	11 - 20 Total=11	21 - 30 Total=20	31 – 40 Total=9	41 – 50 Total=7	51 – 60 Total=3	Total (%)
<i>A.baumannii</i>	2	3	5	2	1	0	13 (30)
<i>K.pneumonia</i>	1	0	2	0	0	0	3 (7.5)
<i>P. aeruginosa</i>	2	5	6	4	1	1	19 (42.5)
<i>S. aureus</i>	1	2	3	2	1	0	9 (20)
Percentage	60%	90.9%	80%	88.8%	42.8%	33.3%	100%

Table (5): Association between Pathogen Type and Burn Degree

Degree	<i>P. aeruginosa</i> (%) Total =19	<i>A. baumannii</i> (%) Total =13	<i>S. aureus</i> (%) Total =9	<i>k. pneumonia</i> (%) Total =3
Second	13 (68.4)	9 (69.2)	6 (66.6)	1 (33.3)
Third	6 (31.5)	4 (30.7)	3 (33.3)	2 (66.6)

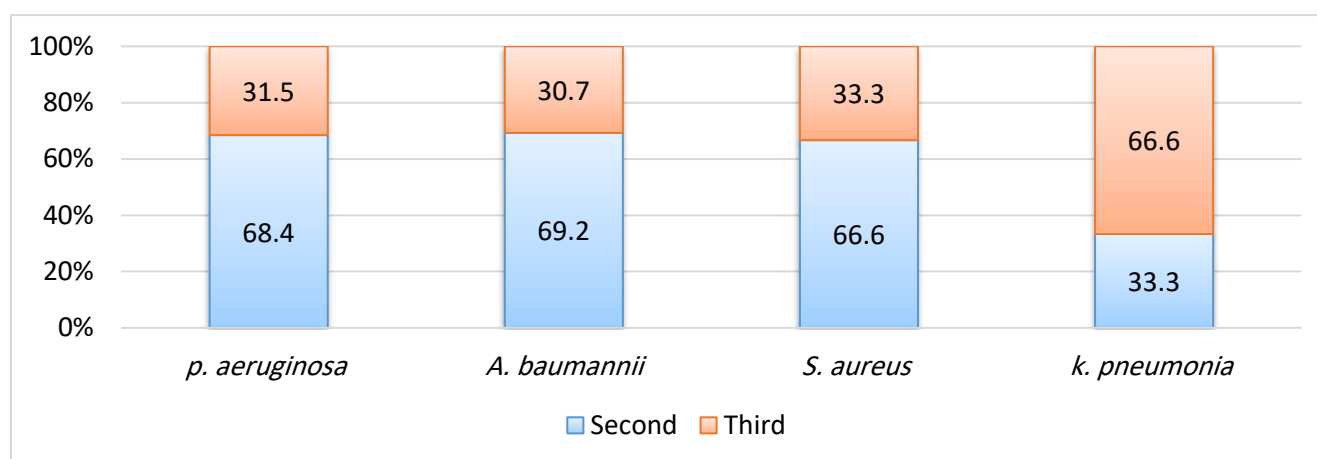


Figure (4): Degree of Burns, Bacteria Types among patient states